MINIREVIEW

Antiretroviral Therapy: Strategies beyond Single-Agent Reverse Transcriptase Inhibition

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INTRODUCTION

In part 1 of this review (23a), agents directed specifically at the inhibition of the human immunodeficiency virus (HIV) reverse transcriptase (RT) were the focus. In this portion, agents directed at other steps in the viral life cycle, both in development and theoretical, as well as immunomodulator approaches and combination strategies will be considered.

RECEPTOR BINDING AND FUSION INHIBITORS

rCD4 and congeners. CD4, a cell surface glycoprotein which is involved in the recognition of class II major histocompatibility molecules by helper T cells, has been identified as the primary receptor for the attachment of HIV to human cells through high-affinity binding with the HIV envelope glycoprotein gp120 (26, 74). Truncated, soluble molecules which contain the extracellular, N-terminal domain of CD4 have been produced by using recombinant DNA technology; these recombinant soluble CD4 (rCD4) molecules maintain their ability to avidly bind HIV gp120 (128). rCD4 has been developed both as a direct antiviral agent and as a targeting molecule, as it can be complexed with toxic substances to guide them specifically to HIV-infected cells which are expressing gp120 on their surfaces. As a direct antiviral agent, it was thought that exposing HIV to an excess of rCD4 might block the virus' ability to bind to cell surface CD4. Additionally, there is evidence that when rCD4 binds to gp120, the gp120 may be stripped away from the virion (105). Theoretically this could interfere with binding to CD4 cells or be directly damaging to the virion. rCD4 has been shown to block acute HIV infection (30, 39, 59, 128, 136) of lymphoid cells as well as monocyte/macrophages (49), and a study with rhesus monkeys demonstrated an effect against simian immunodeficiency virus in vivo (145).

In two phase I human trials of rCD4 administered by intramuscular or intravenous injection to a total of 67 patients in escalating doses as high as 30 mg/day, rCD4 was well tolerated (67, 124) with a serum half-life of the major phase of elimination of approximately 1 h (67). However, a modest, if any, antiviral effect was seen only at the highest dose in one of the trials, despite peak levels in serum in the range previously shown to have in vitro efficacy (30, 39, 59, 128, 136).

In an attempt to increase the serum half-life of rCD4, it has been linked to the heavy chain of immunoglobulin G to create a hybrid molecule termed an immunoadhesin. This

compound, CD4-Ig, also has the potential advantage of crossing the placenta and of increasing immune recognition of the CD4-Ig-HIV complex through binding to the Fc receptors of immune cells. CD4-Ig has been shown to retain the antiviral properties of rCD4 (20, 137) and to mediate antibody-dependent cell-mediated immunity against HIV-infected cells (18). It has been shown that rCD4 and CD4-Ig block syncytium formation between uninfected lymphoid cells and cells infected with HIV type 1 (HIV-1) but that only CD4-Ig blocks syncytium formation mediated by HIV-2 infection in vitro (126).

Extended phase I trials of CD4-Ig are ongoing (22, 27, 55, 149). Preliminary results of these escalating dose studies have confirmed that this molecule is safe and that the predicted longer half-life (32 h in one study) (55) and higher levels in serum are achievable. Antiviral and clinical efficacy of CD4-Ig have not yet been demonstrated, however.

One important concern regarding the potential of rCD4 and its congeners as an anti-HIV strategy and a possible explanation for the lack of more impressive results in trials to date is the demonstration of differences in sensitivity to rCD4 among different strains of HIV. It has been shown that rCD4 has a lower affinity for binding HIV-2 (104) and it has recently been recognized that blocking in vitro infectivity of patient isolates of HIV-1 may require as much as 200- to 2,700-fold-greater concentrations of rCD4 than previously demonstrated with laboratory strains (25). The reason for this difference has been under intense study. The CD4 binding of gp120 derived from rCD4-resistant isolates may be retained, but that of intact virions has been reported to be diminished (13, 104a). In addition, resistant virions may demonstrate decreased gp120 shedding following interaction with rCD4 (104a). This observation emphasizes the importance of testing fresh clinical isolates in addition to laboratory strains for antiviral agent sensitivity. Clinically, this problem might be overcome with higher doses of intravenous CD4-Ig. Alternatively, the in vitro synergy observed with RT inhibitors suggests that CD4-Ig might have a role in combination antiretroviral therapy despite the lack of proven efficacy as a single agent to date (63, 64).

A number of theoretical hurdles to the use of rCD4 and its congeners are worth noting. (i) There has been the possibility that rCD4 interferes with normal CD4 cell function, but this has not been shown in either in vitro or in vivo studies (46, 67, 91, 124). (ii) The described phenomenon of antibody-dependent enhancement of HIV infection via Fc receptor binding (57, 133) has raised the concern that CD4-Ig might mediate this process; however, this has not been found (18). Independent of the issue of antibody dependence, rCD4 has been shown to enhance simian immunodeficiency virus

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infection of a human T-cell line (146) and to be capable of augmenting HIV-2 syncytium induction (126); the mechanism of this phenomenon has not yet been defined but probably involves the exposure of fusogenic domains following the interaction of CD4 with the viral envelope glycoprotein (3). (iii) Another concern with the use of rCD4 molecules has been the possibility of patients forming antibody against the rCD4 protein. Anti-CD4 antibodies have been shown to occur naturally in HIV-infected patients (79), and new antibodies have been detected in a few patients during trials of rCD4 (67, 124); their clinical significance is uncertain. (iv) It is unclear whether gp120 circulates freely in the blood, but there has been concern that if it did, gp120 might bind to the CD4 receptors of uninfected cells, and then these cells might be targeted by rCD4 therapy. It appears that while naturally occurring antibodies may bind gp120 at several sites, the CD4-gp120 interaction is specific to one site, so that gp120 bound to cell surface CD4 is not recognized by soluble CD4. Studies of rCD4 and CD4-Ig therapy have not shown decreases in CD4 lymphocytes with the initiation of therapy (22, 27, 55, 67, 124, 149), suggesting that nonspecific targeting is not a problem.

One goal of antiretroviral therapy is to find a method of selectively killing HIV-infected cells. As noted above, another potential use of the rCD4 molecule is to target such cells. Hybrid molecules have been produced by linking rCD4 domains to toxins such as *Pseudomonas* exotoxin or ricin. In theory, the CD4 portion of the hybrid molecule would bind to infected cells which are expressing gp120 on their surface, and the toxin portion would kill the cell. A number of these molecules have been shown to have activity in vitro (10) without significant toxicity for uninfected cells. One limitation of single-agent therapy with such molecules is that they are only effective against cells which are actively expressing viral gp120 on their surfaces as a result either of productive infection or antigen presentation. In this regard, a CD4-*Pseudomonas* exotoxin molecule has been shown to demonstrate strong in vitro synergy with zidovudine (AZT)—an observation that may prove clinically relevant for the design of future antiviral strategies (5). A phase I trial of a CD4-*Pseudomonas* exotoxin preparation is imminent.

Dextran sulfate and other sulfated polyanions. A series of sulfated polysaccharide, polyanionic compounds have been demonstrated to block the binding of HIV to its receptor (7). Among these compounds are dextran sulfate, heparin, and pentosan, with the first of these having generated the most interest and been the most intensively studied. Recent data suggest that the antiviral effect of dextran sulfate may result from an interaction of this molecule with positively charged amino acids in the V3 loop region of gp120 (the principal neutralizing domain) (19). Dextran sulfate does not significantly inhibit the binding of rCD4 to infected cells expressing gp120 on their surface (19). Interestingly, some clinical isolates of HIV-1 have been shown to have decreased sensitivity to the effects of dextran compared with cloned isolates and the laboratory strain IIB (16). Low-molecular-weight dextran showed additive or synergistic antiretroviral effects in combination with dideoxynucleosides, but antagonism was seen between high-molecular-weight dextran and dideoxynucleosides for two patient isolates (15). A phase II and III trial of dextran sulfate taken orally reported little clinical or virologic effect (1); this negative result may have resulted from the subsequently demonstrated negligible oral absorption (92). However, enthusiasm for this agent has waned further with the recent report that continuous intravenous infusion of dextran sulfate administered to 10 indi-

viduals with symptomatic HIV infection resulted in increased HIV p24 antigen levels and thrombocytopenia in 8 of 10 subjects and alopecia in 5 (39a).

Plant lectins. A series of D-mannose-specific plant lectins have been reported to interfere with an early step of binding or fusion that is different from the effect of the sulfated polysaccharides (9). These compounds do not block the binding of gp120 to the CD4 receptor and are not competitive with dextran sulfate in vitro. Further study is necessary to elaborate the mechanism of action of these molecules.

POST-REVERSE TRANSCRIPTION EVENTS: RNA DEGRADATION, INTEGRATION, TRANSCRIPTION, AND TRANSLATION

RNase H and integrase inhibition. The HIV *pol* gene encodes a number of enzymatic functions related to reverse transcription. First, a DNA polymerase (the RT) assembles a DNA strand complementary to the viral RNA. Then the RNA strand must be removed to allow a complementary DNA strand to form double-stranded DNA. The C-terminal region of the HIV RT enzyme contains a ribonuclease, RNase H, which serves this function. Mutations in the RNase H domain of the RT have been shown to abolish infectivity (135). A putative crystal structure for this enzyme which may lead to the design of drugs to block its function, perhaps by conformational complementarity or by attacking several metal ion-binding sites identified in this molecule, has recently been described (29). The HIV RT molecule also contains an endonuclease or integrase function that is responsible for the integration of the double-stranded proviral DNA into the host cell genome. This is an obvious target for antiviral intervention, but one which remains largely theoretical at the present time.

Transcription and translation inhibition. (i) **Antisense oligonucleotides.** Oligonucleotides which are complementary to a specific segment of a viral RNA genome or mRNA transcript can be synthesized, and by hybridizing with that segment, these oligonucleotides interfere with its normal expression or function. These antisense oligonucleotides may be directed against viral replication by interference with transcription of specific regulatory or structural genes and/or translation and splicing of mRNA.

It has been demonstrated that antisense oligonucleotides may inhibit the replication of HIV in vitro (151), but the first generation of these oligonucleotides was thought to have limited therapeutic potential because of problems with poor permeability into target cells and poor stability because of degradation by host nucleases. It was subsequently demonstrated that phosphate-modified oligonucleotides such as the phosphorothioate derivatives had improved stability and permeability. A number of these analogs have been shown to decrease the replication of HIV-1 and HIV-2 in in vitro systems in the millimolar range (2, 93, 94, 130). Specific targets of effective molecules have included segments of the *rev* and *gag* genes. A methylphosphonate antisense oligonucleotide directed against an HIV enhancer element in the U3 region has been shown to inhibit induction of chronic HIV infection in vitro (89). Many issues remain to be resolved before these agents may be applied therapeutically; among these are the problems of drug delivery and the potential toxicity if these molecules interfere with normal host gene functions.

(ii) **Ribozymes.** A related approach follows from the recognition of a class of RNA molecules called ribozymes,

which possess enzymatic, self-cleaving activity (123). A ribozyme may bind to a specific target RNA segment and catalyze a phosphodiester bond cleavage reaction which does not consume the ribozyme. Potentially, a single ribozyme molecule could act upon many substrate molecules. A number of ribozymes have been isolated from plant RNA pathogens (123) and a family of ribozymes with a "hammerhead" conformational structure has been shown to recognize specific sites on HIV RNA and to cause catalytic cleavage at these sites in cell-free systems. Hammerhead ribozymes have been synthesized with alterations in chain size and structure to improve catalytic function. Again, the potential problems in development as a therapeutic agent include the need to deliver sufficient amounts of stable ribozyme and to show that the ribozyme-HIV RNA interaction is specific enough to avoid toxicity to host RNA. One future solution to the problem of delivery may be the use of gene therapy. An anti-*gag* ribozyme gene has been cloned in a mammalian expression vector and transfected into CD4⁺ HeLa cells (123) with resultant production of the ribozyme without apparent cellular toxicity. When acutely infected with HIV, the ribozyme-producing HeLa cell system showed decreased viral production relative to that by control cells. Examination of cleaved protein species and measurements of proviral DNA suggested that the ribozyme may have cleaved incoming viral RNA (123).

An additional potential problem for the development of effective antisense oligonucleotides and ribozymes is the high mutation rate of HIV, which could lead to changes in the target regions and decreased recognition by these directed molecules. Ideally, however, these compounds would be targeted at critical, conserved regions of the HIV genome where any mutation would be likely to be lethally damaging to viral replication.

(iii) **Tat and Rev inhibitors.** Within the context of the complex genome of HIV, two regulatory genes, *tat* and *rev*, are especially attractive as antiviral targets because of the critical roles played by their protein products in amplifying and directing viral replication. The *tat* gene encodes an essential, 86-amino-acid protein which binds to the TAR site in the HIV long terminal repeat region and affects transcriptional and posttranscriptional processes; its major function may be to facilitate RNA elongation (37). Most interestingly, Tat, as a soluble, *trans*-acting protein, may affect not only the cell in which it is produced but other infected cells as well (41). The *rev* gene produces an essential, 116-amino-acid protein which acts in a *cis* fashion on the Rev-responsive element in the *env* region to enhance the transport of unspliced and singly spliced HIV mRNA from the nucleus to the cytoplasm (121). Strategies to attack these regulatory elements might include the use of antisense oligonucleotides, ribozymes, ineffective mimics of the regulatory proteins which bind to and block the responsive regulatory site but do not cause activation, inhibitors of protein processing which block the splicing of the regulatory gene products, monoclonal antibodies or vaccines directed against the soluble Tat protein, or other compounds which inhibit expression of these regulatory genes or their protein products. Inhibitors of *tat* or the Tat product are in development. One such candidate compound, RO 24-7429, a benzodiazepine derivative, appears promising and has entered a phase I trial, but the future developmental direction for this agent is uncertain (109).

MATURATION AND RELEASE INHIBITORS

IFN- α . Shortly after the discovery of HIV, alpha interferon (IFN- α) was shown to inhibit the replication of HIV in vitro (54). Although it has long been postulated that IFN's action against HIV occurred at a late stage in the replicative cycle, it has only recently been demonstrated experimentally (129). In this study, treatment of chronically infected human lymphoid cell lines produced an antiviral effect, as evidenced by decreases in supernatant p24 antigen and RT activity, but did not significantly decrease the amount of intracellular viral proteins produced. Electron microscopy showed few budding particles and no intracellular particles, supporting the concept of IFN as a blocker of viral assembly and release (129). The *vpu* gene product is known to be important for late-stage events, but the effect of IFN did not vary between *vpu*⁺ and *vpu* mutants in this study. Such studies do not rule out the possibility that IFN may also affect early events in the HIV life cycle, but to a lesser extent.

IFN- α was shown in the early stages of the AIDS epidemic to have a role in the treatment of Kaposi's sarcoma (32, 44, 84, 86), and in some of the patients tested an antiviral effect was demonstrated as measured by reductions in serum p24 antigenemia (32, 86). In a placebo-controlled trial of intravenously administered IFN- α in 34 patients with asymptomatic HIV infection, IFN demonstrated an antiviral effect as measured by decreased ability to culture HIV from peripheral blood mononuclear cells (7 of 17 became culture negative) and a moderate clinical benefit in delaying progression to AIDS (85). However, IFN therapy was also associated with significant toxicity, including granulocytopenia, elevated liver enzymes, and flu-like symptoms, which led to the withdrawal from study of 35% of the IFN group (85). Thus, as a single agent, the role of IFN as an antiretroviral agent is limited, but there is renewed interest in this agent as part of combination regimens (see below).

Recent papers from Kenya (75, 108) have reported dramatic improvements in symptoms associated with the use of daily, low-dose oral IFN- α , with loss of antibody to HIV in some patients. Such remarkable findings are difficult to accept given the dose and route of administration employed and the experience with parenteral IFN to date. It is unlikely that these results will be confirmed, but such studies are planned.

Protease inhibitors. The HIV *pol* gene encodes a protease enzyme which is required for posttranslational cleavage of a precursor p55 Gag protein into four structural core proteins, and for cleavage of the p160 Gag-Pol precursor into RT, endonuclease, structural proteins, and the HIV protease itself (60). The HIV protease is a dimer which is classified as an aspartate protease; human enzymes in this family include renin and pepsin. It has been shown that a frameshift mutation in the proteinase region of the *pol* gene can prevent the processing of the Gag protein precursor (80). Subsequently it was shown that intact HIV protease function is required for the production of mature infectious virions (76). The protease has been sequenced (115), the three-dimensional structure has been determined (107, 148), and the crystalline structure of a protease-substrate inhibitor complex has been described (101). The identification of this virus-specific enzyme required for the production of infectious virions has created great interest in this protein as a possible target for antiretroviral therapy.

A series of HIV protease inhibitors has been designed on the principle of the transition-state mimetic concept (120). Synthetic peptidomimetic compounds which bind to the

protease but whose scissile amide bonds are replaced by nonhydrolyzable elements such as phosphinic acid, reduced amide, hydroxyethylene, or hydroxyethylamine have been synthesized. Such compounds have shown potent anti-HIV activity in the nanomolar range in vitro without cytotoxicity at effective levels (4, 33, 96, 97, 120). Peptide inhibitors have the potential for causing toxicity by interfering with human proteases, but in vitro studies have shown that a number of these compounds may bind to the HIV protease selectively. Other potential problems of peptide-based inhibitors include poor oral bioavailability because of digestion or binding to gut proteases and rapid metabolism. One modification, based on the symmetric homodimeric structure of retroviral protease, was to synthesize a symmetric twofold inhibitor which has reduced peptidic characteristics with potent, specific anti-HIV activity (10,000-fold greater activity against HIV protease compared with related aspartate proteases) and improved stability to degradative enzymes (34). A representative of this class of compounds (115) has entered clinical trials in Europe.

Another approach to protease inhibition has been to look for nonpeptide inhibitors by a structure-based, computer-assisted screening of compounds for potential complementarity with the HIV protease (31). One such compound identified was bromperidol. The related compound haloperidol was shown to inhibit the proteases of HIV-1 and HIV-2 in a selective fashion. The concentrations required were far too high to allow clinical use, but the results suggest that nonpeptide protease inhibitors might theoretically be developed.

The HIV Vif protein is known to be important for full infectivity of the HIV virion (38). Recently it has been reported that Vif may be a protease in the cysteine protease family, which is involved in processing the viral envelope protein, specifically gp41 (47). The sequence of the Vif protein suggests structural homology with known cysteine proteases, and Vif-dependent function has been reported to be impaired by a known inhibitor of cysteine proteases (47). This suggests an additional avenue for future development of therapeutic protease inhibitors.

Glycosylation inhibitors. Cellular enzymes also participate in posttranslational modification of viral proteins through processes such as glycosylation and myristoylation. Because these processes are mediated by cellular rather than virus-specific enzymes, the development of potent but nontoxic therapeutic compounds has been difficult. The trimming glucosidase inhibitors castanospermine and *N*-butyl-deoxynojirimycin have been shown to block HIV infectivity in vitro (45, 70, 116, 143) and to block syncytium formation when HIV-producing and uninfected cells are mixed (70, 116). *N*-Butyl-deoxynojirimycin has entered phase I trials.

Myristoylation inhibitors. Myristoylation inhibitors which block the addition of myristic acid to HIV Gag proteins (which is important for proteolytic processing and viral assembly) have been identified. A number of these agents were shown to decrease productive HIV infection in vitro, but clinical development of these agents was slowed by significant cellular toxicity. Recently, a series of myristate analogs in which a methylene group is replaced by a sulfur or oxygen atom has been shown to inhibit acute and chronic HIV-1 infection in H9 cells without cellular toxicity at effective drug levels (15).

Hypericin. Hypericin and pseudohypericin are aromatic polycyclic diones isolated from the plant family Hypericaceae (St. John's wort). Hypericin is thought to impair the assembly and release of mature HIV virions (100). The mechanism

is unclear; hypericin may have direct effects on the HIV virion or may interact with the budding virus at the cell membrane. Hypericin has also been reported to be an inhibitor of protein kinase C (132). Human trials of herbal extracts containing hypericin have not shown a clear benefit. A phase I trial of an intravenously administered synthetic hypericin compound is underway.

MISCELLANEOUS

GLQ 223 and TAP 29. GLQ 223 (compound Q) is a purified preparation of trichosanthin, a plant extract which has been used in China as an abortifacient and anti-trophoblastic tumor agent. It has ribosome-inactivating properties, but its exact mechanism of action against HIV remains to be elucidated. It has been shown to inhibit the replication of HIV in vitro and in samples of patients' blood cocultured with monocyte/macrophages and may selectively kill infected macrophages (95). The in vitro antiviral effect has been reported to be quite prolonged after a single exposure (95). Early trials in humans have shown significant toxicity, most notably neurologic, including dementia and coma (17, 68). Virologic data are limited from these early dose-finding studies. One study reported decreases in p24 antigenemia and increases in CD4 lymphocyte counts in some patients, but the degree of change was modest (17, 141). TAP 29 is a protein also derived from *Trichosanthos kirilowii* that is related to GLQ 223 and exhibits similar anti-HIV activity in vitro but is nontoxic at concentrations 100-fold higher than the 50% inhibitory dose (58).

Pentoxifylline. Tumor necrosis factor (TNF) has been identified as one of a number of cytokines that can upregulate HIV in vitro, and its elevation in patients with HIV infection has been postulated to be an important host factor in potentiating viral replication in vivo (40, 114). Whether HIV, in turn, can induce TNF production is debated (102). Pentoxifylline, a xanthine derivative, has been shown to inhibit HIV expression in vitro and is thought to work through inhibition of TNF (36); clinical trials of this compound are ongoing. Approaches to antiretroviral therapy that involve interference with cellular or immunologic responses that potentiate HIV replication are interesting and unique but simultaneously pose obvious risks to normal host functions.

***N*-Acetyl-cysteine and glutathione.** In a laboratory model of HIV latency, chronically infected cell lines which express low levels of HIV, such as the monocytic U1 cell line, may be induced to produce high levels of HIV by such inducers as TNF- α , phorbol myristate acetate, and interleukin-6 (IL-6). In vitro pretreatment with *N*-acetyl-cysteine, and to a lesser extent with glutathione, suppresses the stimulation of HIV expression by these inducing agents (69); these findings offer another theoretical area for drug development.

IMMUNOMODULATION

A major parallel direction in the field of antiretroviral therapy is the development of agents or techniques which will enhance or restore the host's immune system. The strategies employed here include the use of recombinant cytokines, chemical stimulants of the immune system, cellular or humoral immune reconstitution, and vaccination. As the focus of this review is on specific antiviral chemotherapeutic agents, these approaches will be mentioned only briefly since they are important in this context for their

potential as future components of combination therapies of established HIV infection.

Cytokines. Cytokines, as critical signalling compounds in the immune repertoire, have been the subject of intense interest as potential therapies in the setting of HIV infection. IFNs for their antiviral as well as immunomodulatory properties, ILs for their immunostimulatory and hematopoietic effects, and the colony-stimulating factors for their hematopoietic stimulatory activity have all been investigated in laboratory and clinical studies. Theoretically, these compounds could restore immune deficits, have intrinsic antiviral activity, synergize with specific antiviral agents, or ameliorate the toxicity of other agents. As single agents, IFN- α has shown some promise as noted above, while IFN- γ and IL-2 have thus far given mixed results (52, 81, 106, 140). IL-3 is under study as an agent to improve HIV-associated leukopenia, thrombocytopenia, and anemia. The colony-stimulating factors have been used with some success in ameliorating drug toxicity (43, 113). Imreg-1, a leukocyte-derived extract with immunostimulatory activity, mediated in part by enhanced endogenous cytokine production, has been reported to exhibit some immunologic and clinical benefit in patients with AIDS-related complex (42).

Chemical immune stimulants. Ampligen is a mismatched, double-stranded RNA molecule [poly(I):poly(C₁₂-U)_n] which may enhance immune function through a variety of postulated mechanisms including promotion of IFN-like antiviral activity (21). It may also have direct anti-HIV activity acting at an early stage in viral replication (103). Initial clinical trials of intravenously administered ampligen suggested beneficial effects (21, 131), but doubt remains concerning this agent's true efficacy.

Diethyldithiocarbamate (ditiocarb or DTC) is a drug which has been reported to have beneficial effects in patients with HIV infection but whose mechanism of action is unclear. It possesses antioxidant and chelating activity, inhibits prostaglandin E₂ production, and appears to affect T-cell differentiation (53, 118). Its effects on HIV replication *in vitro* are debated, but DTC probably has little direct antiviral effect. It has improved the immune response of newborn and aged mice and mice treated with chemotherapy or irradiation and has had a beneficial effect in a murine retrovirus model (53). It has also been postulated to have potential direct effects against opportunistic infectious pathogens (118). In a multicenter, double-blind, placebo-controlled trial of 389 patients with AIDS or AIDS-related complex and less than 500 CD4 lymphocytes per mm³, there were statistically significantly fewer opportunistic infections in the DTC-treated group but no survival benefit and no difference in CD4 lymphocyte counts (53). Interpretation of this study is complicated by the concomitant use of AZT and other drugs. Other smaller, randomized, placebo-controlled trials of DTC have reported improvement in symptoms or decreased progression of disease, but the number of overall events was small (88, 118).

Isoprinosine is classified as an immune stimulator, though the mechanism of action is unclear. A recent placebo-controlled trial of HIV-infected individuals showed decreased progression to AIDS in the isoprinosine group without significant differences in CD4 lymphocyte counts. Further study is ongoing (110).

Thymic humoral factor and thymopentin, a synthetic peptide mimicking the active site of thymopoietin, are under study. Preliminary results of two recent placebo-controlled studies of thymopentin involving a total of 136 asymptomatic or mildly symptomatic patients (23, 134) have indicated

some benefit in maintaining CD4 lymphocyte counts and possibly in slowing disease progression, but final results are awaited.

Cellular and humoral reconstitution. It has been demonstrated *in vitro* that CD8⁺ lymphocytes suppress the production of HIV by infected CD4⁺ lymphocytes (142, 144, 147). A phase I trial is ongoing of autologous CD8⁺ lymphocyte infusions in which these cells are isolated from the blood of an HIV-infected patient, stimulated to replicate *in vitro*, and then infused back into the same patient along with IL-2 therapy. This is an overall somewhat impractical approach, but one that may help identify important immune controls *in vivo*. An even more intense and costly approach involves the performance of bone marrow transplantation in combination with AZT therapy. Despite an initial report of some virologic success with this procedure (56), subsequently published experience has not provided support for this approach as presently conceived (87).

Approaches for passive immunotherapy have included the administration of inactivated sera from asymptomatic HIV-infected individuals shown to have high titers of neutralizing and/or p24 antibody (61, 71-73). Improvements in plasma viremia and p24 antigenemia, more so in earlier stages of disease, have been demonstrated in these early studies (73). Clinical efficacy will need to be demonstrated in larger, systematic trials.

Vaccination of HIV positive individuals. The rationale for this approach involves trying to determine the immune responses to HIV that are protective and then to vaccinate already infected individuals with moieties that will maintain these responses to prevent the inexorable progression of immunodeficiency (122). Approaches for active immunotherapy in HIV-infected patients have included immunization with inactivated, envelope-depleted HIV (90), with recombinant envelope proteins such as gp160 (117), recombinant vaccinia-HIV-expressing HIV proteins (24, 111) or immunogenic peptides from HIV Gag, Pol, and Env proteins (111). In a recently reported phase I trial of a baculovirus-expressed HIV gp160 vaccine in patients with early HIV infection and CD4 counts of >400/mm³, 19 of 30 individuals responded to the vaccine as defined by development of gp160-specific humoral and cellular (lymphocyte proliferative) immune responses. Responders had higher entry CD4 counts, a greater number of immunizations, and stabilization of their CD4 counts over the 10 months of the study compared with nonresponders; in contrast, nonresponders experienced a 7.3% fall in CD4 count. Quantitative viral markers were unchanged, and the only toxicity noted was a local reaction at the injection site (117).

COMBINATION THERAPY

The goals for combination antiretroviral therapy are to find agents which are additive or synergistic in their activities, to minimize toxicity, and, hopefully, to decrease the risk of antiviral resistance. The major approaches to combination therapy under evaluation at present include (i) two nucleoside RT inhibitors or a nucleoside RT inhibitor with a non-nucleoside RT inhibitor, (ii) a nucleoside RT inhibitor with an antiretroviral agent acting at another step in the viral life cycle, (iii) an RT inhibitor with an immunomodulator, (iv) an RT inhibitor with a hematopoietic growth factor added to moderate toxicity. Because of the proven efficacy and strategic importance of RT inhibitors, most combination trials include AZT or another RT inhibitor. Although definitions have varied among published reports, *in vitro* studies

have shown a synergistic or at least an additive effect for such combinations as AZT with agents which affect binding such as rCD4 (63) and dextran sulfate (138); non-nucleoside RT inhibitors such as phosphonoformate (35, 77) and nevirapine (119); the trimming glucosidase inhibitor castanospermine (66); immunomodulators such as IFN- α (50), granulocyte-macrophage colony-stimulating factor (48), and amphotericin (103); and targeted toxin therapy such as CD4-*Pseudomonas* exotoxin (5). While AZT is the classic RT inhibitor tested in such studies, similar results are now being reported with other dideoxynucleosides such as dideoxyinosine (51, 65). Additionally, there is interest in three-drug regimens containing AZT, as these may have advantages over two-drug combinations. For example, the three-drug combination of AZT, rCD4, and IFN- α has shown such activity in vitro (64), and a clinical trial of this combination is under way.

Dideoxycytidine (ddC) is being tested in regimens which alternate a week or a month of ddC therapy with a week or a month of AZT therapy, hoping to reduce the toxicity of each agent (12, 127). A study of AZT alternating weekly with ddC showed that the total cumulative dose of ddC that could be given without development of neuropathy was at least fivefold greater when ddC was given in an alternating, rather than a continuous, regimen. Individual patients have been followed on an alternating schedule for over 2.5 years without neuropathy (14, 150). In a pilot study of 15 pediatric patients, ddC was tested in an alternating schedule with AZT, with some patients demonstrating improvements in CD4 lymphocyte counts and decreases in p24 antigenemia. Mouth sores were common, but neuropathy was not seen in the short term on the alternating AZT-ddC regimen (112).

Concurrent administration of nucleoside analogs is the subject of intense interest presently. For example, ACTG 106, a phase I and II dose-finding study of the combination of ddC and AZT in 56 patients with advanced HIV disease has been reported to show maintenance of CD4 levels at the higher dose combinations (98, 99). This therapy is being examined more formally in ACTG 155, in which the combination of ddC (0.75 mg every 8 h) and AZT (200 mg every 8 h) is being directly compared with ddC (0.75 mg every 8 h) or AZT (200 mg every 8 h) alone in patients with CD4 counts of $<300/\text{mm}^3$ who had been receiving AZT for ≥ 24 weeks.

The issue of monotherapy versus combination nucleoside therapy is also being addressed in two large trials. One, the Delta trial in Europe, is examining the combinations of AZT (200 mg three times a day [t.i.d.]) and dideoxyinosine (200 mg twice a day [b.i.d.]) versus AZT (200 mg t.i.d.) and ddC (0.75 mg t.i.d.) versus AZT alone (200 mg t.i.d.) in patients with CD4 counts of $<350/\text{mm}^3$ who are stratified for prior AZT use. The second, ACTG 175, will enroll 2,100 patients in a four-arm study which will compare AZT alone (200 mg t.i.d.) versus ddI alone (200 mg b.i.d.) versus AZT (200 mg t.i.d.) and ddI (200 mg b.i.d.) versus AZT (200 mg t.i.d.) and ddC (0.75 mg t.i.d.) in patients with CD4 counts between 200 and $500/\text{mm}^3$. This trial plans to use a 50% fall in CD4 count as the primary efficacy endpoint and will also stratify patients for prior dideoxynucleoside exposure. Taken together, ACTG 155, the Delta trial, and ACTG 175 will have a great impact on defining the role of combination versus monotherapy for patients with both asymptomatic and symptomatic HIV infection in the years to come.

The combination of AZT with the non-nucleoside RT inhibitor foscarnet has been studied in six patients with AIDS or AIDS-related complex, five of whom remained p24 antigenemic despite AZT therapy (62). During a 2-week

period in which foscarnet was coadministered with AZT, serum HIV p24 antigen levels fell by a mean of 53% and one patient demonstrated a 100-fold decrease in plasma virus titer. This small study demonstrated that additive antiviral activity can be demonstrated in vivo with the combination of two RT inhibitors and lends virologic support to the ongoing or planned combination trials of AZT with other nucleoside or non-nucleoside RT inhibitors.

IFN- α has been shown to exhibit synergistic anti-HIV activity with AZT and has been administered with AZT as combination therapy for Kaposi's sarcoma (78, 82, 83). These studies demonstrated that the combination may be tolerated in some patients with decreases in p24 antigenemia, HIV culture positivity and tumor growth at doses of IFN less than would generally be required when used as a single agent. Substantial toxicity, including granulocytopenia, anemia, thrombocytopenia, increased liver enzymes, and fatigue, was seen at the higher doses of each agent. Similarly, in 18 patients with advanced HIV infection, 17 of whom did not have Kaposi's sarcoma, the combination of IFN- α and AZT did result in a measurable antiviral effect in patients who could tolerate the therapy, but systemic and hematologic toxicities limited treatment in the majority of subjects (11). A colony-stimulating factor such as granulocyte-macrophage colony-stimulating factor is one recent option that can be added to this combination to ameliorate the hematologic toxicity, although cytokine-related side effects such as fever and myalgias may be increased in such regimens (28, 43, 113). Alternatively, IFN may be combined with other RT inhibitors (such as ddC or dideoxyinosine) or other antiretrovirals which have less bone marrow toxicity.

Examples of other combinations in active or planned clinical trials include AZT with IL-2 (125), AZT with other IFNs, AZT with amphotericin, AZT with rCD4 or CD4-Ig, AZT with thymopentin or thymic humoral factor, and AZT with *N*-butyl-deoxynojirimycin.

Combinations of antiretroviral agents are not always predictably additive or synergistic in their activity, and thus a note of caution needs to be interjected. For example, the combination of AZT and ribavirin, an antiviral agent which interferes with posttranscriptional processing of HIV, was found to be antagonistic, probably related to ribavirin's inhibition of AZT phosphorylation (139). Ribavirin was subsequently found to antagonize the effectiveness of a series of other pyrimidine dideoxynucleosides but to enhance the effect of purine nucleosides in vitro (6, 8).

CONCLUSION

The era of antiretroviral therapy is only approximately 5 years old, but already substantial advances in drug development have been achieved. For the foreseeable future, RT inhibitors will remain the centerpiece of the treatment of HIV infection, but the progress seen to date in understanding the molecular biology of HIV and the intense effort in drug development have made agents effective at other points in the viral life cycle and combination therapies realistic possibilities. This, combined with hoped-for progress in our understanding of viral pathogenesis and immunity, should lead to comprehensive and more effective approaches to the management of individuals with established HIV infection at all stages of disease.

ACKNOWLEDGMENTS

S.M.H. is supported by Public Health Service grants AI27659 and HL43510 from the National Institutes of Health and by Defense Department contract DAMD-17-90-C-0106.

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